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Brief Research Communication

No Association Detected Between a D_3 Receptor Gene-Expressed Variant and Schizophrenia

Leelach G. Rothschild, Judith Badner, Anibal Cravchik, Elliot S. Gershon, and Pablo V. Gejman
Clinical Neurogenetics Branch, National Institute of Mental Health, Bethesda, Maryland

A missense polymorphism (glycine to serine) in the first exon of the dopamine D_3 (DRD3) gene was examined in a sib-pairs schizophrenia collection by the transmission test for linkage disequilibrium (TDT). No association due to linkage disequilibrium was detected using TDT. Additionally, no evidence for excess homozygosity was found. © 1996 Wiley-Liss, Inc.*

KEY WORDS: schizophrenia, dopamine D_3 receptor gene, DRD3, polymorphism, transmission for linkage disequilibrium test, TDT

INTRODUCTION

Genetic factors are thought to be involved in the predisposition for schizophrenia. This is supported by family, twin, and adoption studies [Gottesman and Shields, 1982]. The specific genetic mechanisms, however, remain unknown.

Five dopamine receptor genes (D_1, D_2, D_3, D_4, D_5) have been identified in the human genome [for review, see Sibley and Monsma, 1992]. The D_3 dopamine receptor (DRD3) gene is structurally related to the D_2 and D_4 [Van Tol et al., 1991]. Since DRD3 binds antipsychotic drugs with high affinity [Sokoloff et al., 1992; Van Tol et al., 1991] and is mainly expressed in the limbic areas of the brain, which are suggested to play a role in the control of cognitive and emotional aspects of behavior [Schwartz et al., 1993], it seems reasonable to test whether this gene is involved in the pathophysiology of schizophrenia.

Recently, a polymorphism predicting a glycine-serine substitution at position 9 at the N-terminal extracellular domain of DRD3 was reported [Lannfelt et al., 1992]. This polymorphism can be easily studied by digestion of amplified DNA with the restriction endonuclease enzyme Bal I. Bal I detects three restriction sites, 2 constant and one variable (or polymorphic). The presence of 3 Bal I restriction sites defines allele 2 (A2) whereas the absence of the variable Bal I restriction site defines allele 1 [A1; Lannfelt et al., 1992]. Excess of homozygosity at this polymorphism in schizophrenia was first reported by Crocq et al. [1992] and subsequently by Mant et al. [1994]. The analyses of Mant et al., which included an extended sample of patients pooled with the original sample from Crocq et al. [1992], supported a particular excess of the 1-1 genotype. Nimgaonkar et al. [1993] reported data suggesting an increased frequency of A1 with patients having a family history of schizophrenia but no difference in the distribution of alleles at the locus. Although Di Bella et al. [1994] did not find an association of DRD3 alleles with schizophrenia, they reported some modest support for an association with delusional disorder (A1 and 1:1 genotype).

Conversely, a number of groups have reported no association of excess homozygosity or with particular alleles of the Bal I system at the DRD3 locus with schizophrenia [Nanko et al., 1993; Sabaté et al., 1994; Laurent et al., 1994; Nothen et al., 1993; Morell, 1993; Saha et al., 1994; Jonsson et al., 1993; Yang et al., 1993]. Although linkage studies between DRD3 and schizophrenia have resulted in negative LOD scores [Sabaté et al., 1994; Nanko et al., 1994; Mitchell et al., 1993; Wiese et al., 1992], Greenberg [1993] has shown that a susceptibility gene, i.e., a gene that is not necessary for the expression of a disease but that increases its risk or alters the course of a disease, may be associated with illness in the absence of detectable linkage (unless the number of pedigrees studied is very large).

With the exception of the study by Macciardi et al. [1994], which did not find an association between schizophrenia and DRD3 alleles, previous association studies used case-control designs. Using case-control studies to investigate an association between a gene and a complex disease may in some cases result in artificial association (or failure to find one) due to population stratification.

We have used the transmission test for linkage disequilibrium [TDT; Spielman et al., 1993] to test for association of DRD3 with schizophrenia. In this test, the

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Address reprint requests to Dr. Gejman, Clinical Neurogenetics Branch, NIMH, 10 Center Dr MSC 1274, Bethesda, MD 20892-1274.

2 chromosomes passed from the parents to the affected child are labeled as "case" and the 2 chromosomes not passed on to the affected child are not considered to be associated with the disease [Spielman et al., 1993]. TDT considers parents who are heterozygous for an allele associated with disease and evaluates the frequency with which that allele or its alternate is transmitted to affected offspring. The 2 chromosomes unassociated with the disease provide an internal control because they are of the same ethnic extraction as the case chromosomes. The TDT tests association as well as linkage, and is thus less likely to give false positives due to population stratification than case-control association studies and it can be used to test proposed allele associations. Although this test cannot directly detect excess homozygosity, it can detect whether a particular allele is being nonrandomly transmitted.

Forty-four families with one or more offspring affected with DSM-III-R schizophrenia were studied. Forty-one families identified at the Clinical Neurogenetics branch of the National Institute of Mental Health (NIMH) were initially diagnosed using RDC criteria [DeLisi et al., 1987; Gershon et al., 1988] and rediagnosed subsequently by DSM-III-R. An additional 3 families were recently collected using the same methods.

Genomic DNA was extracted from blood lymphocytes and immortalized cell lines. Polymerase chain reactions (PCR) were carried out as described by Lannfelt et al. [1992]. An aliquot (50 μl) of the PCR product was digested for 5 hours using 6 U per sample of the BalI isoschizomer MscI (New England Bio Labs, Beverly, MA). To ensure that all samples were fully digested, they were incubated overnight at 37°C with an additional 6 U per sample of MscI. Samples were subsequently analyzed by electrophoresis in 1% agarose. Two investigators independently scored all genotypes without previous knowledge of diagnosis of individuals.

Results from TDT did not support an association between schizophrenia and the DRD3 MscI polymorphism (Table I). Although a P value of less than 0.08 may be viewed as a trend toward association, it should be noted that the threshold for significance is in this case 0.017, not 0.05, since we performed 3 different analyses. Given the sample size of 71 parent-affected offspring transmissions, the TDT would have detected nonrandom transmissions at a corrected alpha of 0.017 if an allele was transmitted greater than 64% of the time (compared with the expectation of it being transmitted 50% of the time).

Under the null hypothesis of no association and no linkage, the TDT is independent for each member of the pedigree [Spielman et al., 1983] and therefore families with 2 or more affected children do not need to be considered separately. TDT results when only one affected person from each pedigree is considered are not significant ($\chi^2 = 0.81$, df = 1, $P = 0.37$) but there is considerably lower power to detect a significant result since the number of observed transmissions is decreased from 71 to 31.

We also tested for an excess of homozygosity. Genotypes were counted from the family pedigrees and the proportion of homozygotes to heterozygotes was used to determine a χ^2 value. Our first test considered only offspring that had 2 heterozygous parents. Ten families (those with heterozygous parents), containing 17 affected offspring, were analyzed for an excess of homozygosity. We also tested for an excess of homozygotes by systematically choosing one affected offspring (the affected offspring furthest to the left of each pedigree in every case) from each pedigree. The proportion of homozygous offsprings with schizophrenia was not found to be significantly increased in either test (Tables II, III). The test to detect excess homozygosity among the 10 matings of 2 heterozygous parents would have been significant at the correct alpha of 0.017 if there had been greater than 79% homozygotes among the affected offspring. There was limited power to detect any smaller increases in homozygosity due to the small sample size.

The distribution of parental genotypes is not significantly different from what is expected under Hardy-Weinberg equilibrium (23 1-1, 31 1-2, 5 2-2; $\chi^2 = 3.84$, df = 2, $P = 0.15$). There also is not evidence for assortative mating of parents with respect to the DRD3 genotype ($\chi^2 = 7.5$, df = 4, $P = 0.11$) for the 26 matings in which both parents are genotyped. However, the sample would have low power to detect assortative mating.

Although it is possible that this sample did not have the power to detect an association due to a gene of relatively small effect, we would like to note that all 3 tests are consistent with each other, in not showing an association with DRD3. Another valid question is if the

TABLE I. Distribution of Allele Frequencies for DRD3 and Schizophrenia ($\chi^2 = 3.17$, $P = 0.08$, df = 1)*

		Nontransmitted alleles	
		1	2
Transmitted alleles	1	57	43
	2	28	10

*There are 71 heterozygote parent-affected offspring combinations. There are 57 homozygous 1,1 parent-affected offspring transmissions and 10 homozygous 2,2 parent-affected offspring transmissions. Of the 71 heterozygous parent-affected transmissions, A1 was transmitted 43 times and not transmitted 28 times. This is not significantly different from what would be expected under the assumption of no linkage or association.

TABLE II. Testing for an Excess of Homozygosity in Affected Offspring With 2 Heterozygous Parents ($\chi^2 = 1.47$, $P = 0.2$, df = 1)*

Genotype	1-1	1-2	2-2
Observed	4	11	2
Expected	4.25	8.5	4.25

*Results of homozygosity analysis when the affected offspring with 2 heterozygous parents were selected. There are 10 parent-affected offspring combinations in which both parents are heterozygous. Of these combinations, 4 offspring have 1-1 genotypes, 11 offspring have 1-2 genotypes, and 2 offspring have 2-2 genotypes. No significant excess homozygosity is observed under the assumption of the Hardy-Weinberg equilibrium. The χ^2 value was obtained by calculating the proportion of homozygotes to heterozygotes.

TABLE III. Testing for an Excess of Homozygosity in One Affected Offspring Per Family (χ^2 = 0.39, P = 0.5, df = 1)*

Genotype	1-1	1-2	2-2
Observed	19	21	3
Expected	20	18	4

* Results of homozygosity analysis when from each pedigree only one affected offspring (the one furthest to left of each pedigree) was selected. By this criterion there were 43 selected offspring. Nineteen offspring have 1-1 genotypes, 21 offspring have genotypes 1-2, and 3 offspring have genotypes 2-2. No significant excess homozygosity was observed under the assumption of the Hardy-Weinberg equilibrium. The χ^2 value was obtained by calculating the proportion of homozygotes to heterozygotes. Selecting from each pedigree the offspring furthest to the right resulted in similar results (data not shown).

whole DRD3 gene can be excluded as a susceptibility locus by a single TDT test. Although the size of the DRD3 gene is relatively small, the chromosomal area excluded by TDT, which corresponds to the linkage disequilibrium area, should also be relatively small, but cannot be delimited with precision with the available information. Mutational analysis of structural and regulatory sequences of the DRD3 gene in a large number of individuals might be necessary to rule out the DRD3 as a candidate gene for schizophrenia.

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